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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is bracketed and inserted material is underlined.

Claims:

- 1. (Amended Three Times) A method for the bioproduction of a C₆ to C₂₂ monoor di-carboxylic acid comprising
- a) contacting, under aerobic conditions, a transformed *Pichia pastoris* [characterized by] <u>comprising</u> a genetically-engineered alkane hydroxylating activity comprising
- i) at least one copy of a foreign gene encoding cytochrome P450 monooxygenase; and, optionally,
- ii) at least one copy of a foreign gene encoding cytochrome P450 reductase, each gene operably linked to a *Pichia pastoris* Aox 1 promoter such that alkane hydroxylating activity is enhanced upon contact with at least one C₆ to C₂₂ straight chain hydrocarbon; and
 - b) recovering the C₆ to C₂₂ mono- and di-carboxylic acids.
- 6. (Amended Two Times) A transformed *Pichia pastoris* strain [characterized by] comprising an enhanced alkane hydroxylating activity and comprising,
- a) at least one DNA fragment from Candida maltosa ATCC 90677 selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37); and, optionally,
- b) at least one DNA fragment from Candida maltosa ATCC 90677 encoding cytochrome P450 reductase, each DNA fragment operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C₆ to C₂₂ straight chain hydrocarbon.
- 8. (Amended Two Times) A method for the enhanced bioproduction of C₆ to C₂₂ mono- and di-carboxylic acids comprising
- a) contacting, under aerobic conditions, a transformed Candida maltosa [characterized by] comprising a genetically-engineered, enhanced alkane hydroxylating activity with at least one C_6 to C_{22} straight chain hydrocarbon, wherein said alkane hydroxylating activity arises from
- i) at least one additional copy of the genes encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716)

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SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)); or

- ii) at least one additional copy of the gene encoding cytochrome P450 reductase (D25327); or
 - iii) at least one additional copy of both the genes of i) and ii); and
 - b) recovering the C₆ to C₂₂ mono- and di-carboxylic acids.
 - 9. (Amended Two Times) The method of Claim 8 wherein
 - (a) the genetically-engineered, enhanced alkane hydroxylating activity arises
- i) at least one additional copy of the genes encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)); or
- ii) at least one additional copy of the gene encoding cytochrome P450 reductase (D25327); or
 - iii) at least one additional copy of both the genes of i) and ii);]
 - [b] \underline{a}) the at least one C_6 to C_{22} straight chain hydrocarbon is dodecane; and
 - [c] b) the product recovered is dodecanedioic acid.
- 14. (Amended One Time) A method for the enhanced bioproduction of C₆ to C₂₂ mono- and di-carboxylic acids comprising
- a) contacting, under aerobic conditions, transformed Candida maltosa [characterized by] comprising a genetically-engineered, blocked β -oxidation pathway with at least one C_6 to C_{22} straight chain hydrocarbon, wherein the β -oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase; and
 - b) recovering the C₆ to C₂₂ mono- and di-carboxylic acids.
- [15. The method of Claim 14 wherein the transformed Candida maltosa β-oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.]
- 16. (Amended Two Times) A transformed Candida maltosa [characterized by] comprising disruption of no more than both POX4 genes encoding acyl-CoA oxidase whereby a β-oxidation pathway is functionally blocked.
- 17. (Amended One Time) A transformed Candida maltosa [characterized by] comprising a β-oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase using a single URA3 selectable marker.
- 19. (Amended One Time) A method for the enhanced bioproduction of C₆ to C₂₂ mono- and di-carboxylic acids comprising

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a) contacting, under aerobic conditions, transformed Candida maltusa [characterized by] comprising.

i) a genetically-engineered, enhanced alkane hydroxylating activity, wherein the enhanced alkane hydroxylating activity arises from

- 1) at least one additional copy of a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)), or
- 2) at least one additional copy of a gene encoding cytochrome P450 reductase (D25327 (SEQ ID NO:43)), or
- 3) at least one additional copy of both the genes i) and ii), and ii) a genetically-engineered, blocked β-oxidation pathway, [with at least one C₆ to C₂₂ straight chain hydrocarbon] wherein the β-oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase; and
 - b) recovering the C₆ to C₂₂ mono- and di-carboxylic acids.
- 25. (Amended One Time) [A] <u>An isolated DNA fragment comprising a</u>) a first Candida maltosa promoter operably linked to a gene encoding a Candida maltosa cytochrome P450 monooxygenase and b) a second Candida maltosa promoter operably linked to a gene encoding a Candida maltosa cytochrome P450 reductase.
- 26. (Amended Two Times) [A] An isolated DNA fragment comprising a) a first Candida maltosa PGK promoter which is operably linked to a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41), and Alk8 (D12719 (SEQ ID NO:42)) and b) a second Candida maltosa PGK promoter operably linked to a gene encoding a Candida maltosa cytochrome P450 reductase.